REMARKS

Reconsideration of the above-identified application in view of the amendments above and the remarks following is respectfully requested.

Claims 16-51 are in this case. Claims 19, 20, 23, 24, 29, 30, 33, 34, 37, 38, 41, 42, 46, 47, 50 and 51 have been withdrawn from consideration as being drawn to a non-elected invention. Claims 16-18, 21, 22, 25-28, 31, 32, 35, 36, 39, 40, 43-45, 48 and 49 have been rejected. Claims 18, 21, 22, 28, 31, 32, 36, 39, 40, 45, 48 and 49 have now been cancelled. Claims 16, 17, 25, 35 and 43 have now been amended.

35 U.S.C. § 112, First Paragraph, Rejections

The Examiner has rejected claims 6-18, 21, 22, 25-28, 31, 32, 35, 36, 39, 40, 43-45, 48 and 49 under U.S.C. § 112, first paragraph, as containing subject matter that was not described in specification in such a way as to reasonably convey to one skilled in the relevant art that the inventors, at the time the application was filed, had possession of the claimed invention. The Examiner's rejections are respectfully traversed. Claims 18, 21, 22, 28, 31, 32, 36, 39, 40, 45, 48 and 49 have now been cancelled, rendering moot the Examiner's rejections thereof. Claims 16, 17, 25, 35 and 43 have now been amended.

The Examiner states that "all of the current claims encompass a genus of nucleic acids which are defined solely by function, not disclosed in the specification", that "*no common element or attributes of the sequences are disclosed*", and that "*no* structural limitations which provide guidance on the identification of sequences which meet these functional limitations is provided".

Contrary to the Examiner's contention, it is the Applicant's strong opinion that the instant specification and original claims provide support for the primers, probes and DNA chips identifying polymorphic SSRs for the characterization of *Listeria monocytogenes* strains, as taught in the present invention.

In particular, the Examiner has stated that claim 16 is drawn to a genus of primers which comprises many hundreds of trillions of different possible sequences, while "the applicant has expressed possession of, at most, 42 different sequences which amplify such repeats". In support of this rejection, the Examiner quotes the decision from The Regents of the University of California vs. Eli Lilly and Co 43

USPQ2d, stating that the current claims name a type of material which is generally known to exist, without providing knowledge of the material composition. Further, the Examiner quotes the decision from Fiers vs Sugano 25 USPQ2d, stating that the current situation is a definition of a compound solely by it's functional utility, without any definition of any particular element that is common to all species of the genus.

Applicant is of the strong opinion that the production and use of primers, probes and DNA chips identifying polymorphic SSRs for the characterization of *Listeria monocytogenes* strains, as taught in the present invention, are clearly defined in the instant specification, and that the decisions brought by the Examiner in support of his rejections are not relevant to the claims under examination.

Specifically, decisions such as The Regents of the University of California vs Eli Lilly and Co and Fiers vs. Sugano relate to genes hybridizing to portions of coding sequences (such as cDNA fragments), which have varying degrees of sequence homology to a specific nucleotide sequence, and encoding a polypeptide having a defined function: "It is only a definition of a useful result rather than a definition of what achieves that result. Many such genes achieve that result" (The Regents of the University of California vs Eli Lilly and Co), and "...if an inventor is unable to envision detailed chemical structure of DNA sequence coding for a specific protein...then conception is not achieved...until after gene has been isolated..." (Fiers vs Sugano). In contrast, the oligonucleotide primers, probes and DNA chips identifying SSRs of the present invention do not represent probes for genes or sequences encoding functional polypeptides, rather sequences capable of amplifying, or binding to, the short simple sequence repeats abundant in the non-coding regions of prokaryotic genomes, for which sequence information is available.

Regarding the question of whether one of ordinary skill would recognize that the inventors...had possession of the claimed invention as claimed, the Guidelines for Examination of Patent Applications states that:

"The written description requirement for a claimed genus may be satisfied through sufficient description of a representative number of species by actual reduction practice..., or by disclosure of relevant identifying characteristics, i.e., structure or other physical and/or chemical properties, by functional characteristics

coupled with a known or disclosed correlation between function and structure, or by a combination of such identifying characteristics..."

and that:

"What constitutes a "representative number" (of examples of reduction to practice of members of a genus) is an inverse function of the skill and knowledge in the art. Satisfactory disclosure of a "representative number" depends on whether one of skill in the art would recognize that the applicant was in possession of the necessary common attributes or features of the elements possessed by the members of the genus in view of the species disclosed."

The instant specification provides both detailed description of methods for identification of SSRs in prokaryotic genomic sequences (see, for example, Genomic Sequence Analysis in Experimental Methods, page 43) and examples of reduction to practice of primers for amplification of such SSRs, using the genome sequence of *E. coli* as a representative prokaryote genome (see Table 2, page 52). Further, abundant SSRs from the genomes of a large variety of additional prokaryotic and one simple eukaryotic (*S. cerevesia*) species were identified using the methods described (see Table 1, pages 48-51). Since the number of SSRs found present in the 11 surveyed genomes is so vast (see, for example, Table 3), there are, as the Examiner notes, a myriad of possible oligonucleotide primers, probes and DNA chips which could be used to identify SSRs in the genomes of *Listeria* strains. However, considering the methods commonly available in the art, such as the published software tool described in the instant specification, genomic DNA sequences of *Listeria* sp. available from GenBank, and common protocols for synthesizing probes, DNA chips and oligonucleotide primer sequences, and their common chemical structure, one of ordinary skill in the art would recognize that primers, probes and DNA chips for identification of *Listeria* SSRs can be produced as easily as those described for *E. coli*. Thus, Applicant believes that, contrary to the Examiner's contention, the specification describes the claimed invention in such a way as to fulfill the written description requirements of possession.

While reducing the present invention to practice, it was observed that a total of 12.1 to 31.6 percent of the 11 genomes assessed is comprised of SSRs (Table 3), that the overwhelming majority of hypervariable SSR arrays are comprised of mono-

and dinucleotide repeats (Table 1 and Table 2), and that SSR polymorphisms are concentrated in the 5' and 3' flanking regions of genes, and not within the coding sequences (see page 53, lines 1-8, and Table 2). Thus, independent claims 16, 25, 35 and 43 have now been amended to further include the limitations "polymorphic mono- or dinucleotide simple sequence repeat loci", "...wherein said polymorphic simple sequence repeat locus comprises at least four nucleotides in at least one of it's polymorphs..." and "whereas said polymorphic simple sequence is in a non-coding region of said...genome", thereby further limiting and defining the scope of the claimed primers, probes and DNA chips to those identifying mono- and dinucleotide SSRs in the non-coding regions of the *Listeria* genome.

Support for such amendments can be found throughout the instant specification, for example:

> ***SSRs in E. coli:***
>
> A computerized scan of the genome of *E. coli* revealed many small arrays of SSRs. Of 199,766 loci with simple sequence repeats (Table 1, top panel), 191,563 exhibited mononucleotide, 6,363 dinucleotide, 2,069 trinucleotide, 48 tetranucleotide, and 2 hexanucleotide core repeat length. These SSRs were distributed rather evenly throughout the genome (Figure 1a). They are mostly located in non-coding areas, with the exception of those with 3 or 6 bp core sequences, which often were located in coding areas. Since the *E. coli* genome does not contain long non-coding sequences, most SSR arrays in non-coding areas were immediately upstream or immediately downstream of a gene, often in locations where variability presumably might affect gene expression (Kashi et al. 1997, King et al. 1997, Kashi and Soller 1998)."(page 49, line 33 to page 50, line 9; see also Table 2).

and

> "In all genomes, the distribution of core repeat lengths was skewed toward mononucleotides. All genomes

exhibited a distribution of total array lengths that also tended toward low numbers, three tandem repeats in most genomes. However, *Bacillus subtilis* exhibited a relatively small proportion of loci where mononucleotides where tandemly repeated three times.

The number of genomic sites exhibiting longer array lengths differed among genomes. All genomes showed 99.99 % of SSR arrays at 12 or fewer bp in length. Total SSR content of the genomes varied from 12.1 to 31.6 percent, with most genomes clustered toward the middle of the range. SSRs tended to comprise a larger proportion of smaller genomes than of larger ones (Table 3)."(page 54, line 26, to page 55, line 3)

and

"Variation of mononucleotide SSRs among strains of *E. coli* was confirmed by sequencing the PCR product for the SSR and flanking domains of all nine SSR loci. Variations of DNA sequence at polymorphic loci are shown in Figure 3. Results of DNA sequencing confirmed that the SSR arrays were hypervariable, exhibiting several alleles for copy number. Additionally, point mutations in sequences flanking the SSR arrays at both loci were the results of expansion or deletion of tandem mononucleotide repeats. The SSR polymorphisms were located just upstream of open reading frames."(page 53, lines 1-9)

and

"polymorphic mononucleotide sites that were found in *E. coli* exhibit 1 to 4 bp size differences. These small numbers of repeats are well suited for the development of

SSR allel -specific oligonucleotides (ASOs)."(page 18, lines 5-8)

Thus, the primers, probes and DNA chips of the present invention, as described in amended claims 16, 25, 35 and 43, identify short, abundant mono- and dinucleotide SSRs found in the non-coding regions of the *Listeria monocytogenes* genome.

In view of the evidence and arguments brought hereinabove Applicant believes to have successfully overcome the 35 U.S.C. § 112, first paragraph rejections.

35 U.S.C. § 102 (b) Rejections - Rasmussen, et al.

The Examiner has rejected claims 6-18, 21, 22, 25-28, 31, 32, 35, 36, 39 and 40 under 35 U.S.C. as being anticipated by Rasmussen et al. (Microbiology, 1995;141:2053-2061). Claims 18, 21, 22, 28, 31, 32, 36, 39 and 40 have now been cancelled, rendering moot the Examiner's rejection thereof. Claims 16, 17, 25 and 35 have now been amended. The Examiner's rejections are respectfully traversed.

The Examiner states that Rasmussen et al. teach a series of PCR primers and probes which amplify SSR regions in *Listeria monocytogenes*, and that the PCR products formed by Rasmussen et al. will inherently function as allele-specific probes and are allele-specific oligonucleotides.

Applicant wishes to point out that the Rasmussen et al. reference relates to the characterization of patterns of conserved amino acid sequence types in different serotypes of *Listeria monocytogenes*. The authors sequenced genomic DNA from three genes in a variety of *L. monocytogenes* strains, using specific oligonucleotide primers, and observed a high degree of sequence conservation, even within the areas of nucleotide differences:

> "Within the repeat region, no strain-specific nucleotide differences were found within the groups of the sequence type 1 or the sequence type 3 strains, respectively, whereas few strain-specific differences were found among the sequence type 2 strains" (page 2055, right column, third paragraph)

and

> "*L. monocytogenes* has been shown to exist in at least three well-conserved evolutionary lines with a strong degree of conservation within those lines. The majority of the isolates group into two of the sequence types for which there is a correlation with flagellar antigens as well as grouping based on MLEE, RAPD, RFLP and PFGE" (page 2060, left column, second paragraph).

In stark contrast, the primers, probes and DNA chips for identifying the polymorphic mono- or dinucleotide simple sequence repeat loci of the present invention are based on the observation that these SSRs comprise hypervariable regions which are not restricted by environmental selection pressure as are the amino acid sequences found in the coding regions of the genes investigated by Rasmussen et al. (see above).

The Examiner further states that the primers amplify the iap gene (Figure 1, page 2059) which shows the presence of several repeats, including an AAAAA monomer repeat in the non-coding region, and that the PCR products would inherently be allele specific oligonucleotides. Careful examination of the sequence data presented in Figure 1 of the prior art document (page 2059) shows that the AAAA (and not AAAAA) repeat cited by the Examiner is not within a non-coding region, but rather outside of the 400 bp region specific for *L. monocytogenes* (see page 2055, left column, second paragraph). The nucleotide sequence appearing in the referenced sequence (type 2 line, last row, figure 1, page 2059) contains the codons CTT (leu), GGA (gly), AAA (lys) and GCT (ala), representing amino acid coordinates 379-382 of the iap (strain EGD) coding sequence (see NCBI Accession No. AF532302). Such sequences located outside of the 400 bp *L. monocytogenes* specific sequence of the iap coding region apparently lack nucleotide polymorphisms, as emphasized by the authors:

> "Finally, a 99 bp region downstream of the *L. monocytogenes* specific region of Iap was sequenced for six strains of type 1 and one type 2 strain. All sequences in this region were

identical to that of strain EGD" (page 2057, left column, second paragraph).

Independent claims 16, 25, and 35 have now been amended to further include the limitations "polymorphic mono- or dinucleotide simple sequence repeat loci", "...wherein said polymorphic simple sequence repeat locus comprises at least four nucleotides in at least one of it's polymorphs..." and "whereas said polymorphic simple sequence is in a non-coding region of said...genome", thereby further limiting and defining the scope of the claimed primers, probes and DNA chips to those identifying short, polymorphic mono- and dinucleotide SSRs in the non-coding regions of the *Listeria* genome.

The cited repeat sequence is not located within a non-coding region, nor does it represent a polymorphic, and certainly not hypervariable, region. Similarly, the other repeat sequences cited by Rasmussen et al. represent portions of the coding sequence of iap, and are, by nature, also not found in a hypervariable region. Thus, oligonucleotide primers and probes specific for the repeat sequences taught in Rasmussen et al. would not, and could not, function as allele-specific probes of the present invention, as stated by the Examiner. As such, Rasmussen et al. fails to teach, and would not motivate one of ordinary skill in the art to make or use the primers, probes and DNA chips identifying polymorphic mono- and dinucleotide SSRs in the non-coding regions of the *Listeria* genome recited in amended independent claims 16, 25 and 35.

35 U.S.C. § 103 (a) Rejections- Rasmussen, et al. in view of Gingeras et al.

The Examiner has rejected claims 43-45, 48 and 49 under U.S.C. 103(a) as being unpatentable over Rasmussen et al. (Microbiology, 1995;141:2053-2061) as applied to claims 6-18, 21, 22, 25-28, 31, 32, 35, 36, 39 and 40 and further in view of Gingeras et al. (U.S. Pat. No. 6,228,575). Claims 18, 21, 22, 28, 31, 32, 36, 39, 40, 45, 48 and 49 have now been cancelled, rendering moot the Examiner's rejection thereof. Claims 16, 17, 25 and 35 and 43 have now been amended. The Examiner's rejections are respectfully traversed.

The Examiner states that Rasmuss n et al. do not teach diagnosis of *L. monocytogenes* using DNA chips, and that Gingeras et al. teach a method of detecting *Listeria* by detecting nucleic acids on a DNA chip.

Gingeras et al. teach the preparation, methods for use and computer programs for DNA chips comprising oligonucleotide arrays representing organism-specific nucleotide sequences, which can be used for determination of groups or species by comparison of hybridization patterns of nucleic acids from the organisms. Such DNA chips are described in the instant specification (see pages 18 and 19, and the section titled "DNA Chip Design" and Table 4 in the Examples section of the instant specification). However, Gingeras et al. do not disclose DNA chips comprising probes and primers useful in identifying different strains of *L. monocytogenes*.

Applicant wishes to point out, as is detailed hereinabove, that all the repeat sequences cited by Rasmussen et al. represent portions of the coding sequence of the iap gene, and are, by nature, not found in a hypervariable region. Thus, oligonucleotide primers and probes specific for the repeat sequences taught in Rasmussen et al. would not, and could not, function as the primers or probes for use in DNA chips identifying polymorphic mono- and dinucleotide SSRs in the non-coding regions of the *Listeria* genome as recited in amended independent claim 43. As such, it is Applicant's strong opinion that now amended independent claim 43, and all claims depending therefrom, are not anticipated by, nor rendered obvious thereby by Rasmussen et al. in combination with Gingeras et al.

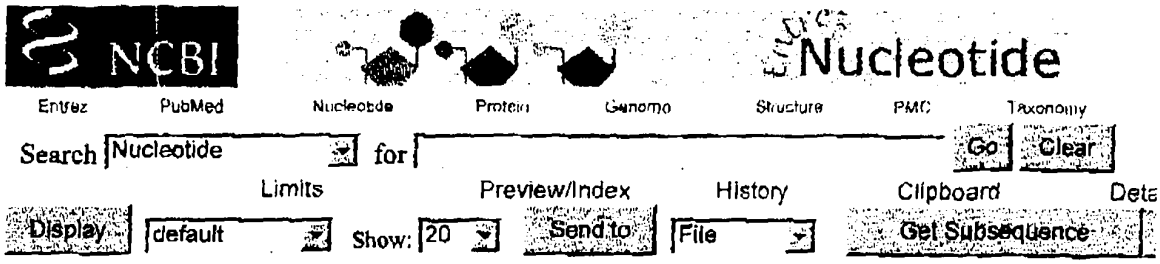
In view of the abov amendments and remarks it is respectfully submitted that claims 16, 17, 25, 27, 35, 43 and 44 are now in condition for allowance. Prompt notice of allowance is respectfully and earnestly solicited.

Respectfully submitted,

Sol Sheinbein
Registration No. 25,457

Date: October 26, 2003.

Encl.:
A three months extension fee
NCBI Acc. No. AF532302



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REFERENCE   1  (bases 1 to 1449)
  AUTHORS   Schmid,M., Walcher,M., Bubert,A., Wagner,M., Wagner,M. and
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  TITLE     Nucleic acid-based, cultivation-independent detection of Listeria
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  JOURNAL   FEMS Immunol. Med. Microbiol. 35 (3), 215-225 (2003)
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